Allosteric Modulation of Drug Binding to Human Serum Albumin

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Abstract: Human serum albumin (HSA), the most prominent protein in plasma, is best known for its extraordinary ligand binding capacity. The three homologous domains of HSA (labeled I, II, and III), each in turn composed of two subdomains (named A and B), give rise to the three-dimensional structure of HSA. This flexible structural organization allows the protein structure to adapt to a variety of ligands. As conformational adaptability of HSA extends well beyond the immediate vicinity of the binding site(s), cooperativity and allosteric modulation arise among binding sites; this makes HSA similar to a multimeric protein. Although kinetic and thermodynamic parameters for ligand binding to HSA calculated by quantitative structure-activity relationship models are in excellent agreement with those obtained *in vitro*, cooperative and allosteric equilibria between different binding sites and competition between drugs or between drugs and endogenous ligands make difficult the interpretation of HSA binding properties *in vivo*. Binding of exogenous and endogenous ligands to HSA appears to be relevant in drug therapy and management. Here, the allosteric modulation of drug binding to HSA is briefly reviewed.

Keywords: Human serum albumin, Drug binding, Structure, Allostery, Structure-activity relationship, Biomedical aspects.

INTRODUCTION

Human serum albumin (HSA) is the most prominent protein in plasma (its concentration being 45 mg/mL, *i.e.* 7.0×10^{-4} M, in the serum of adults), but is also found in tissues and secretions throughout the body. HSA is best known as a volume expander, providing a depot for many compounds so they will be available in quantities well beyond their solubility in plasma. Indeed, HSA shows an extraordinary binding capacity for various ligands including amino acids (Trp and Cys), metal ions, thyroxine, and bilirubin. HSA has a high affinity for heme and is responsible for the transport of lipophilic compounds and drugs and of medium and long chain fatty acids (FAs) [1-9].

HSA abundance makes it an important determinant of the pharmacokinetic behavior of many drugs. In other cases, HSA holds some ligands in a strained orientation, favoring their metabolic modification, and renders potential toxins harmless, by transporting them to disposal sites. HSA also accounts for most of the antioxidant capacity of human serum, directly, or by binding and carrying radical scavengers, or by sequestering transition metal ions with pro-oxidant activity. Furthermore, HSA acts as a nitric oxide depot and displays enzymatic properties [2,6,7,10-15].

Binding to HSA is also a means for developing contrast agents for magnetic resonance imaging endowed with high intravascular retention (blood pool agents), such as those used in the clinical practice for visualizing vascular structures (magnetic resonance angiography; MRA) and for detecting regions with abnormal vascular permeability [16,17]. Last but not least, heme-HSA mutants have been proposed as oxygen carriers not only for red blood cell substitutes but also as O_2-therapeutic reagents [18,19].

HSA is constituted by a single non-glycosylated all-α chain of 65 kDa containing three homologous domains (labeled I, II, and III). Each domain is known to be made up by two separate helical subdomains (named A and B), connected by random coils. Terminal regions of sequential domains contribute to the formation of interdomain helices linking domain IB to IIA, and IIB to IIIA, respectively (Fig. **1**) [5-9,20,21].

HSA-LIGAND RECOGNITION

The HSA globular domain structural organization provides a variety of ligand binding sites (Fig. **1**). A partial list of site-specific HSA ligands is summarized in Table **1** (see [22]). Bulky heterocyclic anions bind preferentially to Sudlow's site I (located in subdomain IIA), whereas Sudlow's site II (located in subdomain IIIA) is preferred by aromatic carboxylates with an extended conformation (*e.g.*, ibuprofen), benzodiazepines (*e.g.*, diazepam), and some anesthetics [2,5,7,22,23].

HSA is able to bind up to seven equivalents of long chain FAs at multiple binding sites (labeled FA1 to FA7) (Fig. **1**) with different affinity. In sites FA1-FA5 the carboxylate moiety of FAs is anchored by electrostatic/polar interactions; on the contrary, sites FA6-FA7 do not display a clear evidence of polar interactions that keep in place the

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carboxylate head of the FA, thus suggesting that sites FA6-FA7 are low-affinity FA binding sites. Remarkably, FA7 represents Sudlow's site I (*e.g.*, the warfarin site), whereas the cavity hosting FA3 and FA4 contributes to form Sudlow's site II (*e.g.*, the ibuprofen site) [1,4,7,24-28].

FA1, located in subdomain IB (Fig. **1**), hosts the heme, with the tetrapyrrole ring arranged in a D-shaped cavity limited by two tyrosine residues (Tyr138 and Tyr161) that provide π-π stacking interaction with the porphyrin and supply a donor oxygen (from Tyr161) for the ferric heme iron. Ferric heme is secured by the long IA-IB connecting loop that fits into the cleft opening. Heme propionates point toward the interface between domains I and III and are stabilized by salt bridges with His146 and Lys190 residues [29,30].

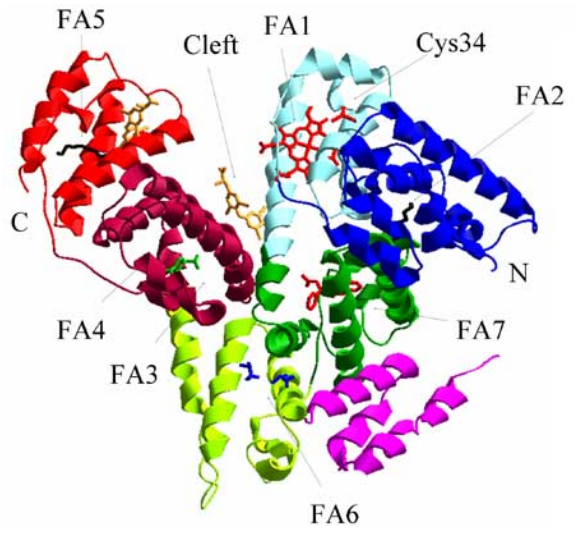

Fig. (1). HSA structure. The six subdomains of HSA are colored as follows: subdomain IA: blue; subdomain IB: cyan; subdomain IIA: dark green; subdomain IIB: yellow-green; subdomain IIIA: purple; subdomain IIIB: red. The heme (in red) fits the primary cleft in subdomain IB, corresponding to FA1. Sudlow's site I (in subdomain IIA, corresponding to FA7) is occupied by warfarin (in yellow). Sudlow's site II (in subdomain IIIA, corresponding to FA3-FA4) is occupied by ibuprofen (dark green). FA6 is occupied by two halothane molecules (in blue). FA2 and FA5 are occupied by myristate anions (in black). Thyroxine (in orange) fits the additional site in subdomain IIIB and the cleft between domains I and III. The Cys34 residue is rendered with red sticks. GA is in magenta. Atomic coordinates were taken from PDB entries 1E7C, 1H9Z, 1HK4, 1O9X, 1TF0, and 2BXG [22,23,25,27,30,31,38]. For details, see Table **1** and text.

Among other ligands, triiodobenzoic acid binds to Sudlow's site I and at the heme cleft together with a myristate anion. Furthermore, in the absence of FAs the thyroid hormone thyroxine primarily binds to Sudlow's site I, though there are three secondary sites in domain III, one that accommodates the diiodophenol ring in Sudlow's site II and two that overlap with FA5. In the presence of FAs saturating all FA binding sites, thyroxine binds to a pocket between domains I and III (Fig. **1**) [31]. Also the general anesthetic halothane binds to Sudlow's site II; additionally, two more copies of the ligand are accommodated within the FA7 cleft, *i.e.* at the interface between subdomains IIA and IIB [27]. Last but not least, Cys34 represents the *S*-nitrosylation site of HSA, this plasma protein representing a NO depot and a NO transducer (Fig. **1**) [12,32,33].

HSA-PROTEIN RECOGNITION

Numerous Gram-positive bacterial species, including human pathogens, express surface proteins that interact with host proteins like HSA [34]. The anaerobic bacterium *Finegoldia magna* is present in the indigenous flora of the skin, the oral cavity, and the gastrointestinal and urogenital tracts. However, these bacteria are also important human pathogens connected with conditions such as soft tissue abscesses and deep wound infections [35]. Some isolates of *F. magna* bind HSA to their surface, and the molecule responsible for this is called protein PAB [36].

Protein PAB contains a domain showing high sequence homology (60%) to the albumin binding domains (ABDs) of protein G, a bacterial protein that binds the FC domain of IgGs [37]. The ABD of protein G (GA) is formed by 53 amino acid residues and is composed of a left-handed three-helix bundle. The GA amino acid residues from the second helix and the loops surrounding it are involved in HSA binding. GA binds to the HSA domain II close to a cleft bounded by helices 2 and 3 in subdomain IIA, helices 7 and 8 in subdomain IIB, and the loop region before helix 7 in subdomain IIB. The hydrophobic core of the HSA-GA interface is lined with residues Phe228, Ala229, Ala322, Val325, Phe326, and Met329 from HSA and residues Phe27, Ala31, Leu44, and Ile48 from GA. The hydrophobic interaction between the HSA residue Met329 and the GA side chain Phe27 may play a major part in the species specificity observed for HSA-ABD complex formation (Fig. **1**) [38].

Adjacent to the hydrophobic core of the HSA-GA interface is a hydrogen bond network between helix 7 in HSA subdomain IIB and the loop preceding helix 2 in GA. HSA residue Glu321 forms two hydrogen bonds, one each with the main chain nitrogens of Thr24 and Ser25 in GA. Furthermore, the side chain hydroxyl group of Ser25 in GA forms another hydrogen bond with the side chain oxygen of Asn318 from HSA. The main chain oxygen atom of Asn318 is, in turn, involved in a hydrogen bond with the side chain hydroxyl group of Tyr28 in the GA module. At the opposite end of the binding interface, a second hydrogen bond network is formed between residues in the loop connecting helices 2 and 3 in GA and residues in helices 2 and 3 in HSA subdomain IIA. HSA residues Glu230 and Asn267 form hydrogen bonds to the main chain nitrogen atom and the side chain hydroxyl group of the GA residue Thr37, respectively. The HSA residue Glu230 also interacts with the main chain oxygen atom of the GA residue Ala35. An additional hydrogen bond at the HSA-GA interface occurs between the GA residue Glu47 in helix 3 and the HSA residue Lys212 (Fig. **1**) [38].

As a whole, although the biological function(s) of the GA module is not known in detail, the acquisition of the GA module seems to add selective advantages to the

Table 1. Site-Specific Ligand Binding to HSA[a]

	Binding site						
	FA1	**FA2**	**FA3-FA4**	**FA5**	**FA6**	**FA7**	**Cleft**
			(Sudlow's site II)			**(Sudlow's site I)**	
Ligand	Heme	Myristate	Ibuprofen	Myristate	Myristate	Warfarin	Thyroxine
	Myristate		Myristate	Thyroxine	Halothane	Myristate	Iodipamide
	Azapropazone		Diazepam	Oxyphenbutazone	Ibuprofen	Thyroxine	
	Indomethacin		Halothane	Propofol	Diflunisal	Iodipamide	
	Triiodobenzoate		Propofol			Triiodobenzoate	
			Indoxyl sulfate			Indomethacine	
			Diflunisal			Phenylbutazone	
			Thyroxine			Oxyphenbutazone	
						Indoxyl sulfate	
						Diflunisal	

[a] From [22].

bacterium in terms of growth and also increases its virulence. In fact, the HSA-GA complex formation could provide growing bacteria with FAs and, possibly, other nutrients transported by HSA. Therefore, compounds inhibiting the HSA-ABD complex formation could be used to treat infections caused by HSA binding bacterial pathogens [38].

ALLOSTERIC MODULATION OF DRUG BINDING TO HSA

The conformational adaptability of HSA involves more than the immediate vicinity of the binding site(s). This is at the root of the observed ligand-dependent allosteric conformational transition(s) (Figs **1** and **2**) [3,6-8,22,39-49].

The heme-binding cleft and Sudlow's site I are functionally linked. In fact, the affinity and the second order rate constant for heme binding to HSA decrease by about one order of magnitude upon drug (*e.g.*, warfarin) binding. According to linked-functions (see Appendix) [50,51], heme binding to HSA decreases the affinity and the second order rate constant for drug (*e.g.*, warfarin) binding by the same extent. On the other hand, kinetics of heme and drug dissociation from HSA complexes is unaffected by third components. This indicates drugs that bind to Sudlow's site I (*e.g.*, warfarin) act as allosteric effectors for heme association and *vice versa* (Fig. **2**). By contrast, the heme binding cleft and Sudlow's site II are functionally uncoupled, thus drug binding to Sudlow's site II (*e.g.*, ibuprofen) does not affect heme association and *vice versa* [43,48,52].

Benzodiazepines bind to several functionally and allosterically linked HSA clefts, depending on their optical conformation and substitution. Allosteric interactions have been reported to affect stereoselective binding equilibria between HSA and ibuprofen, warfarin, and lorazepam. In particular, binding of either ibuprofen enantiomers to HSA affects the interaction mode of lorazepam. Moreover, lorazepam binding to HSA affects the binding mode of warfarin enantiomers [41,47,53]. Furthermore, allosteric effects have been reported for *S*-oxazepam hemisuccinate/*R*-oxazepam hemisuccinate, ibuprofen/*S*-lorazepam acetate, and L-tryptophan/phenytoin binding to HSA [54,55]. Carbamazepine shows direct competition with L-tryptophan by binding to Sudlow's site II, but allosteric interactions occur with ligands that bind to Sudlow's site I, the tamoxifen cleft, and the digitoxin site [56].

Remarkably, NO, N_2O, halothane, and chloroform bind to HSA cleft(s), such as Sudlow's site I, which appears spectroscopically- and allosterically-linked to Cys34. It is noteworthy that NO can modify HSA structure without nitrosylation of the Cys34 residue. These findings suggest that NO may modulate anesthetic binding to HSA and support the hypothesis that some physiological effects of NO result from anesthetic-like non-covalent bonding to proteins [12,32,33].

Fatty acids (FAs) are effective in the allosteric regulation of the HSA binding properties, especially of Sudlow's site I. Myristate regulates the HSA binding properties in a complex manner, involving both competitive and allosteric mechanisms. First, Sudlow's site I ligands (*e.g.*, warfarin) displace FAs bound at site FA7, and site Sudlow's II ligands (*e.g.*, ibuprofen) displace FAs bound at sites FA3 and FA4. Accordingly, heme binding to HSA displaces FA bound at site FA1. As concerns conformational changes at the basis of allosteric modulation of binding properties, FAs binding essentially determines a relative domain rearrangement at the I-II and II-III domain interfaces. This allosteric regulation is not observed for short FAs (*e.g.*, octanoate) that preferably bind to Sudlow's site II and displace the specific ligands (*e.g.*, ibuprofen) without inducing HSA allosteric rearrangement(s). This indicates that

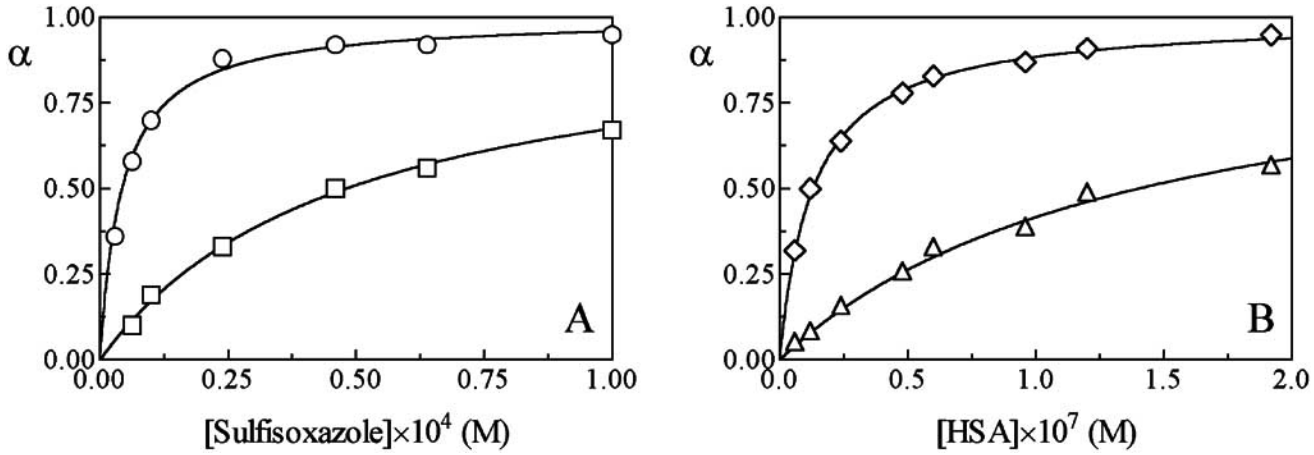

Fig. (2). Allosteric modulation of drug binding to HSA. (Panel A) Sulfisoxazole binding to HSA in the absence (circles) and presence (squares) of the heme. (Panel B) Heme binding to HSA in the absence (diamonds) and presence (triangles) of sulfisoxazole. The continuous lines were calculated according to the following equation:

$$\alpha = ([L]/\{K^{-1} + [L]\} \quad (1)$$

with values of $K_\alpha^{-1} = 4.3\times10^{-6}$ M, $K_\beta^{-1} = 4.8\times10^{-5}$ M, $K_A^{-1} = 1.3\times10^{-8}$ M, and $K_B^{-1} = 1.4\times10^{-7}$ M. α is the molar fraction of the ligand-bound HSA derivative, K (*i.e.*, K_α or K_β or K_A or K_B) is the association equilibrium constant, and [L] is the free ligand concentration (see Scheme 1). According to linked functions (see Scheme 1), $K_\alpha/K_\beta = K_A/K_B = 11$. HSA concentrations are plotted on the x-axis for experimental reasons. This allows to maintain constant the chromofore (*i.e.*, heme) concentration over the whole ligand (*i.e.*, HSA) concentration range explored. Otherwise if heme concentration was plotted on the x-axis, the chromofore (*i.e.*, heme) concentration changed within the HSA binding experiments, impairing data analysis. All data were obtained at pH 7.0 and 25.0°C. For further details, see text. From [48].

the hydrophobic interactions between the long FA polymethylenic tail and HSA drives allosteric rearrangements [23,25,27,30,46,49].

Extensive conformational changes occur following myristate binding to HSA. Actually, addition of up to three equivalents of long-chain FAs is reported to enhance the binding of Sudlow's site I (*i.e.*, FA7) ligands, and this behavior has been explained by a cooperative effect established by FA binding to domain III (*i.e.*, to FA4 and FA5). On the other hand, myristate bound at the limit of subdomain IA (*i.e.*, to FA2) was suggested to be functionally linked to Sudlow's site I. It should be noticed that binding of more than three equivalents of myristate decreases warfarin affinity for Sudlow's site I, as does Fe(III)heme. From the structural viewpoint, the conformation of the subdomain IA is remarkably affected by myristate binding, and seems to be almost unaffected by the presence of additional ligands bound to the myristate-HSA complex. It should be noticed that the IA-IB interdomain interface hosts the heme binding site, thus providing an explanation for the allosteric interaction between heme and FAs. On the other hand, in the myristate-HSA-heme ternary complex the conformation of subdomain IIB is more similar to the conformation of ligand-free HSA rather than that of the myristate-HSA complex, either in the absence or presence of warfarin. Moreover, the long α-helix that connects subdomain IIB to IIIA is remarkably tilted, thus affecting both geometry and stereoselectivity of Sudlow's site II [3,23,25-27,29-31,49,57-59].

FAs binding to HSA might influence the HSA-GA complex formation. In fact, the side chain of the HSA residue Lys212 forms a 3.6-Å salt bridge with the carboxyl group of the HSA side chain Glu208 in defatted HSA. In the presence of three dodecanoate (laurate) ions located in the FA6 and FA7 sites of HSA, Lys212 forms a tighter 2.6-Å ion pair bond to the side chain of the GA residue Glu47 [38].

The thyroid hormone thyroxine binds to Sudlow's sites I and II, and to two sites in subdomain IIIB. Although FAs compete with thyroxine at all four sites, they induce conformational changes that create a fifth hormone-binding site in the cleft between domains I and III. Mutation of residue Arg218 within Sudlow's site I greatly enhances the affinity for thyroxine and causes the high serum thyroxine levels associated with familial dysalbuminemic hyperthyroxinemia (FDH). Structural analysis of two FDH mutants of HSA (Arg218→His and Arg218→Pro) shows that this effect arises because substitution at Arg218, which contacts the hormone bound in subdomain IIA, produces localized conformational changes that relax steric restrictions on thyroxine binding at this site [31,60].

Notably, HSA undergoes pH- and allosteric effector-dependent reversible conformational isomerization(s). Between pH 2.7 and 4.3, HSA shows a fast (F) form, characterized by a dramatic increase in viscosity, low solubility, and a significant loss in the α-helical content. Between pH 4.3 and 8.0 and in the absence of allosteric effectors, HSA displays the normal (N) form which is characterized by the typical heart-shaped structure. Between pH 4.3 and 8.0 in the presence of allosteric effectors (*e.g.*, drugs and long-chain FAs), or at pH greater than 8.0 in the absence of ligands, HSA changes conformation to the basic (B) form with the loss of α-helix and an increased affinity for certain ligands, such as warfarin [39,42,44,45,61,62].

STRUCTURE ACTIVITY RELATIONSHIPS

Very recently, high-throughput methods have been developed to assay the HSA binding properties of compound libraries [63-68]. The set of experimental data can be used for the training of machine-learning methods to predict HSA binding [69-72].

Kinetic and thermodynamic parameters for drug binding to HSA calculated by quantitative structure-activity relationship models are in excellent agreement with those obtained experimentally (see [69-73]). Correlations based on large HSA-ligand binding data sets have highlighted the importance of molecular descriptors (*i.e.*, lipophilicity, acidity, hydrogen bonding potential, and shape factors) in predicting HSA binding properties [67,69-72].

However, cooperative and allosteric equilibria between different binding sites and competition between drugs or between drugs and endogenous ligands make difficult the interpretation of HSA binding properties *in vivo* [2,3,22,41-43,48,49,52-54,74-76]. Therefore, experimental evidences such as structural determination of HSA-ligand complexes and solution studies of allosteric and cooperative modulation of ligand binding to HSA are still a need.

BIOMEDICAL ASPECTS

HSA binds different classes of ligands at multiple allosterically linked binding sites. The non-specific binding of drugs to plasma proteins is an important determinant for their biological action since it modulates drug availability to the intended target. From the thermodynamic viewpoint, the issue of drug availability to the desired target can be formulated as a multiple equilibrium in which a drug is able to bind to various proteins with different binding affinities. Moreover, several drugs can bind to the same protein site or to different functionally linked clefts following competitive and synergistic mechanisms [3,6,13,48,52,69,71-73,76].

Interestingly, for many drugs the fraction bound to HSA can be higher than 90%, which constitutes a non-trivial problem in drug therapy. In fact, the total drug concentration required to achieve the desired action may turn out to be one or even several orders of magnitude higher in the presence of plasma proteins than in their absence. By contrast, the increase of plasmatic levels of endogenous ligands (*e.g.*, ferric heme) under pathological conditions (*e.g.*, hemolytic anemia) may induce a massive release of drugs with the concomitant intoxication of the patient [3,6,13,48,52,69,71-73,76].

The most complex question, which may give rise to unexpected problems, is that the free fraction of a given drug depends on the concomitant use of more drug(s), all binding to HSA. Although competitive displacement of drugs from HSA has long been considered, and numerous drug classes are widely known to pharmaco-kinetically interact by this means, knowledge and detailed information on the complex and reciprocal allosteric modulation of drug binding to plasma proteins is somewhat less diffuse, precise and systematic [3,6,13,48,52,69,71-73,76].

Binding to HSA also affects distribution and clearance of low molecular weight proteins (*i.e.*, less than 30 kDa) and peptides that, when unbound, should be rapidly cleared by the kidneys. HSA acts therefore as a carrier for smaller proteins allowing them to circulate for a time as long as the half-life of HSA itself [77,78]. Therefore, the actual concentration of small proteins (*e.g.*, cytokines) in any compartment depends on the ability of such proteins to bind to HSA and/or other plasmatic carrier proteins [79].

HSA-protein interaction is also a matter of concern in biomarker discovery by plasma proteomics. The large difference between HSA concentration and usual levels of small proteins that could serve as disease markers makes the identification of less-concentrated proteins unaffordable, therefore HSA (50% of the average plasma protein load) and immunoglobulins (25% of the average protein load) are routinely removed from plasma or serum [79,80]. By this procedure, smaller proteins interacting with HSA and other abundant carrier proteins are depleted as well. Mass spectroscopic analysis of human serum following molecular mass fractionation demonstrated that the majority of low molecular weight biomarkers exist bound to carrier proteins [78]. For this reason, HSA could be used to specifically harvest smaller proteins and to enrich the specimens in candidate peptides and proteins with potential diverse tissue and cellular origins that may reflect important disease-related information [78,81].

CONCLUSION AND PERSPECTIVES

The ligand binding affinity for HSA is one of the most important factors affecting the distribution and the free concentration of many administered drugs. While a certain extent of HSA interaction may be desirable to help drug solubilization and distribution, a too tight interaction negatively affects the distribution to sites of action and dramatically raises the total concentration of the administered drug. It appears therefore important that also the general medical practitioner be aware of the extraordinary multiplicity of drug binding properties, and conformational flexibility, of HSA, so that in employing several drugs he adequately considers possible pharmaco-kinetic interactions, even among unrelated - and unsuspected - drug classes. On the other hand, any new drug that appears to bind HSA must be carefully examined with respect to all possible conformational changes it may induce in HSA, and all possible allosteric interactions it may display towards the binding of other - even though fully unrelated - drugs.

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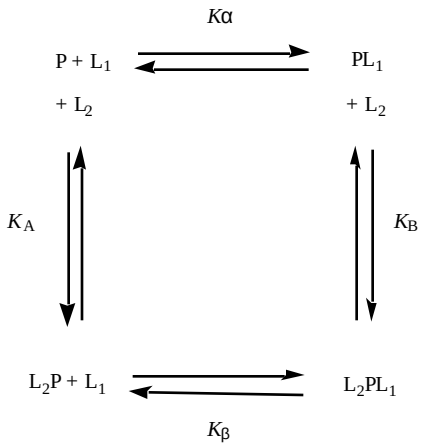
APPENDIX

The reaction mechanism that describes linked functions (Scheme **1**) in terms of association equilibrium constants [50,51] considers a protein, P (*i.e.*, HSA), that can bind two different ligands L_1 (*e.g.*, sulfisoxazole) and L_2 (*e.g.*, heme), each at a distinct binding site (see Fig. **2**). Since each equilibrium is assumed to be rapid and not independent of the others, it follows that [50,51]:



Scheme 1.

$$K_\alpha \times K_B = ([L_2PL_1])/([P]\times[L_1]\times[L_2]) = K_A \times K_\beta \quad (1A)$$

if $K_\alpha < K_\beta$, then $K_A < K_B$; and if $K_\alpha > K_\beta$, then $K_A > K_B$. In other words, thermodynamics requires that if the binding of component L_1 is affected by the addition of component L_2, then there must be a reciprocal effect of added L_1 on the interaction of L_2 with P. Moreover, if the two binding sites are functionally-unlinked, then $K_\alpha = K_\beta$ and $K_A = K_B$.

Taking into account all the species involved in Scheme 1, it is possible to describe the variation of the L_2-dependent apparent equilibrium constant *K'* (between the protein P and the ligand L_1) as well as its reciprocal effect, represented by the change of the L_1-dependent apparent equilibrium constant *K"* (between the protein P and the ligand L_2) by the following equations [50,51]:

$$K' = ([PL_1]+[L_2PL_1])/\{([P]+[L_2P])\times[L_1]\} = K_\alpha\{(1+K_B\times[L_2])/(1+K_A\times[L_2])\} \quad (2A)$$

$$K'' = ([L_2P]+[L_2PL_1])/\{([P]+[PL_1])\times[L_2]\} = K_A\{(1+K_\beta\times[L_1])/(1+K_\alpha\times[L_1])\} \quad (3A)$$

Values of K_α, K_β, K_A, and K_B relevant to Scheme 1 may be easily obtained from plots of *K' versus* $[L_2]$ (according to eqn. 2A) and of *K'' versus* $[L_1]$ (according to eqn. 3A). When P is ligand-free, then $K' = K_\alpha$ (according to eqn. 2A) and $K'' = K_A$ (according to eqn. 3A), and when P is L_2- and L_1-saturated, then $K' = K_\beta = K_\alpha \times K_B/K_A$ (according to eqn. 2A) and $K' = K_B = K_A \times K_\beta/K_\alpha$ (according to eqn. 3A), respectively [50,51].

ABBREVIATIONS

ABD	=	albumin binding domain
FA	=	fatty acid
FDH	=	familial dysalbuminemic hyperthyroxinemia
GA	=	protein G-like albumin binding domain
HSA	=	human serum albumin
MRA	=	magnetic resonance angiography

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